3436-Pos
Is RNA Self-Assembly a Mechanism Used by Cells to Influence Gene Expression?
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RNA molecules are important factors involved in different cellular processes and have a multitude of roles in the cell. These roles include serving as a temporary copy of genes used for protein synthesis or functions in translational machinery. Interestingly, RNA is so far the only biological molecule that serves both as a catalyst (like proteins) and as information storage (like DNA). However, in contrast to proteins well known to be able to self-associate, such polymers have never been reported for natural RNA. We present here evidence that such a polymer of a natural RNA, the DsrA RNA, exists in the bacterial cell. DsrA is a small noncoding RNA (87 nucleotides) of Escherichia coli that acts by base-pairing to mRNAs in order to control their translation and turnover. One of the best-characterized target of DsrA is the rpoS mRNA. This messenger is of primary importance in the bacteria because it encodes the sigma S subunit of RNA polymerase and thus is a major regulator of stress response: the production of sigma S results in dramatic changes in cellular morphology and physiology. The results we obtained about DsrA self-assembly and our assumption about the function of this assembly in the cell will be presented herein.

Protein-Nucleic Acid Interactions III

3437-Pos
ParA2, a Vibrio Cholerae Chromosome Partitioning Protein, Forms Helical Filaments on DNA
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Much less is known about how bacteria segregate DNA prior to replication than is known about such processes in eukaryotic cells. Most bacterial chromosomes contain homologs of plasmid partitioning (par) loci. These loci encode ATPases called ParA that are thought to contribute to the mechanical force required for chromosome and plasmid segregation, but the mechanisms by which these ATPases function in segregation are unknown. In Vibrio cholerae, the chromosome II (chrII) par locus is essential for chrII segregation. Electron microscopy and three-dimensional reconstruction revealed that ParA2 formed bipolar helical filaments on DNA in a sequence-independent manner. These filaments had a distinct change in pitch when ATP was present compared to when either ADP or no nucleotide was present. Fitting recently determined crystal structures of ParA proteins into our ParA2 nucleoprotein filament enabled us to model how ParA2 bound and coated the DNA. Our findings raise the possibility that ParA2-mediated changes in DNA topology could contribute to chrII segregation.

3438-Pos
Structure and Dynamics of the Cytidine Repressor DNA-Binding Domain
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The *E. coli* cytidine repressor (CytR) is a member of the LacR family of bacterial repressors that differentially regulates nine operons. The natural operators in CytR-regulated promoters are comprised of a pair of degenerate recognition half-sites arranged as inverted repeats and separated by a variable length spacer. Spacers range from 0 to 9 basepairs accounting for up to a 25Å translation and 310 degree rotation of the half-sites. Characterizing the interactions between the CytR DNA-binding domain (DBD) and DNA is critical to understanding the mechanism of differential gene regulation. Analysis of the DBD structure using NMR allowed us to assess both the structure and the dynamics of the DBD in relation to DNA sequence specificity. Here, we present the structure of a CytR DBD monomer bound specifically to one DNA half-site of the uridine phosphorylase (udp) operator. We find that the DBD exists as a three-helix bundle containing a canonical helix-turn-helix motif similar to other proteins that interact with DNA. The structure of the DBD in the presence of recognition site DNA reveals a departure in helical orientation from other members of the LacR family. In addition, the DBD structure differs when bound to nonspecific DNA and populates two distinct conformations when free. Nonspecific binding results in measurable changes in protein dynamics when compared to the protein specifically bound to the udp half-site substrate. Thus for CytR, the transition from nonspecific association to specific recognition results in changes in protein mobility that are coupled to structural rearrangements.

3439-Pos
Determining the Role DNA of Bending in Topology Simplification by Type II Topoisomerases
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Type II topoisomerases are essential and universally conserved enzymes that simplify the global topology of DNA by an ATP dependent mechanism that involves passing one double stranded segment (T segment) of DNA through a transient double stranded break in a second segment (G segment) of DNA. This core strand passage mechanism allows type II topoisomerases to decatenate DNA and relax supercoils to below equilibrium levels. Although much is known about the structure and function of these critical proteins, the specific mechanism that drives this topology simplification reaction remains an open question, though several compelling theories have been presented. Some of the more plausible theories postulate that type II topoisomerases achieve non-equilibrium topology simplification by inducing a sharp bend into the G segment. In this study we sought to determine if and to what extent type II topoisomerases impose a bend on DNA. We used Atomic Force Microscopy (AFM) to visualize protein-DNA complexes of three different type II topoisomerases that span the range of topology simplification activity. We directly measured the bend angles imposed on DNA by these proteins to determine if the bend angles could fully account for the observed topology simplification behavior. We found that type II topoisomerases bend DNA, but the measured bend angles were not in accordance with the relative non-equilibrium activity. These findings suggest that bending of DNA could be an important component of the mechanism of topology simplification by type II topoisomerases but it cannot completely account for the observed topology simplification behavior.

3440-Pos
Single-Molecule Study of Human Topoisomerase II
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Topoisomerase II is an essential enzyme that maintains genomic integrity by simplifying global DNA topology through supercoil relaxation, decatenation of linked chromosomal DNA, and DNA unknoting. This is achieved by generating a transient double-stranded break in one DNA segment through which a second double-stranded segment is passed prior to religation of the break. Failure or prevention of DNA religation leads to double stranded DNA breaks and fragmentation of the genome. Thus, the DNA cleavage reaction of human topoisomerase II (hTopo II) is one of the most successful targets for anti-cancer drugs. In order to develop effective anti-cancer drugs, it is critical to understand the detailed mechanism of DNA supercoil relaxation driven by hTopo II. One of the two isoforms of human Topoisomerase II, hTopo IIα, preferentially relaxes positive supercoils, a feature it shares with only one other type II topoisomerase; *E. coli* topoisomerase IV. Here, we have investigated the mechanism of DNA supercoil relaxation by hTopo IIα using single molecule magnetic tweezers experiments. We measured the DNA relaxation rate of positive and negative supercoils and found that positive supercoil relaxation was ~2 times faster than negative supercoil relaxation. This result indicates that the relaxation mechanism of hTopo IIα is different from that of topoisomerase IV, which displays an almost absolute preference for positively supercoiled DNA in single-molecule experiments. Our data further suggest that there is a small, ~30%, difference in the processivity of hTopo IIα relaxing positive versus negative supercoils. These data, together with twist dependent relaxation rate measurements, allow a detailed comparison with the mechanism of chiral discrimination by *E. coli* topoisomerase IV, and suggest possible mechanisms of chiral discrimination by type II topoisomerases.

3441-Pos
Dynamics of DNA-Bending in Binding Site Recognition by IHF
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We present recent progress in monitoring the DNA bending dynamics in site-specific recognition by IHF, an architectural protein from *E. coli* that recognizes several sites on phage λ DNA, primarily by indirect readout. IHF bends the DNA at its cognate site by nearly 180° over ~35 bp, creating two kinks in